SYNTHESIS AND BIODISTRIBUTION OF ACETYL-3,5-BR-82-DIBROMOSALICYLIC ACID*

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SUMMARY

Acetyl-3,5-Br-82-dibromosalicylic acid (dibromoaspirin) was synthesized by radiobromination of salicylic acid to yield 3,5-dibromosalicylic acid, followed by acetylation of this intermediate. Biodistribution studies in mice with and without a neuroblastoma showed that the tumor/blood radioactivity ratio was low (0.3 at one hour). Thin layer chromatography of urine indicated that only 20 to 30% of the radioactivity corresponded to dibromoaspirin. Studies of rabbits with inflammatory lesions showed that the inflammatory/normal tissue uptake ratio was 1.0 to 1.9. The RBC/plasma ratio was less than 0.5. Low uptake of radioactivity was likely due to rapid dehalogenation in vivo.

Key words: Acetyl-3,5-Br-82-dibromosalicylic acid, Dibromoaspirin, Neuroblastoma

INTRODUCTION

Acetyl-3,5-dibromosalicylic acid (dibromo-aspirin) has been shown to acetylate hemoglobin in vitro, thus inhibiting sickling(1). Radiolabeled dibromo-aspirin might thus be useful in various hematological studies. Since aspirin is an anti-inflammatory and antiprostaglandin agent(2), dibromo-aspirin may also possess related pharmacological properties. In order to evaluate the biological behavior of dibromo-aspirin, we have synthesized the radiolabeled precursor, 3,5-Br-82-dibromo-salicylic acid as well as acetyl-3,5-Br-82-dibromo-salicylic acid (Br-82-dibromo aspirin). Biodistribution studies were carried out in mice with and without a neuroblastoma, and in rabbits.

EXPERIMENTAL

The synthesis of 3,5-Br-82-dibromo-salicylic acid was adapted from the procedure for producing the nonlabeled compound(3). Acetylation of the radiolabeled and nonlabeled 3,5-dibromo-salicylic acid was carried out by modif-

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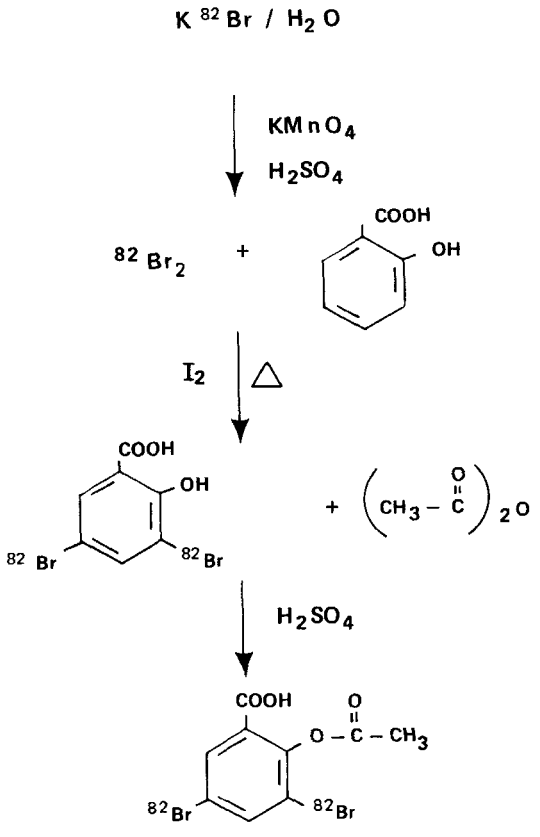

Figure 1. Synthesis of 3,5-Br-82-dibromo-salicylic acid and acetyl-3,5-Br-82-dibromo-salicylic acid.

ication of established procedures (1,3,4).

Generation of bromine-82 by bromination of salicylic acid was carried out by variations of the procedures of Slaunwhite and Neely(5), and Markey, Colburn and Kopin(6). To the bromine-82 generator flask (Slaunshite and Neely(5), 5mCi $K^{82}Br$ was added (obtained from NEN in H_2O, concentration = 1.99 mCi/ml, specific activity=2.47 mCi/mg Br). This was followed by addition of 10 mg of KBr as carrier, 10 drops of conc. H_2SO_4 and 10 drops of 1N $KMnO_4$. Nitrogen was allowed to sweep through the system for about 5 minutes. The generated elemental bromine-82 was frozen on the inside wall of the conical reaction vessels (immersed in a dry ice/acetone mixture). The recovered Bromine-82, with 3 to 4 mCi of activity, was then mixed with 0.5 ml of glacial acetic acid.

Radiobromination of salicylic acid was carried out by mixing a glacial acetic acid solution of salicylic acid (2mg/0.5ml) with the $^{82}Br_2$ / acid mixture, followed by gentle heating. While the solution was still hot, 0.6 mg of I_2 was added. The reaction proceeded for an hour at room temperature. The solution was then heated, and underwent vaccum evaporation. The reaction product was purified by Prep-scale TLC using Silica Gel 60F 254 (E. Merek) and $CHCl_3$: ethanol : acetic acid (27:3:3) as solvent. The R_f, as indicated by an authentic sample of 3,5-Br-82-dibromosalicylic acid (Aldrich Chem., Milwaukee, Wis.) was 0.58. The purified 3,5-Br-82-dibromosalicylic acid was extracted with ethanol for biodistribution studies. The reaction products and purified compound were also analyzed by TLC, using Silica Gel 60F 245 aluminum sheets with a solvent of ethyl acetate/isopropanol/10% ammonia (5:2:1), R_f=0.54, and HPLC with μBondapak C-18 column (Waters Assoc.) with eluant acetonitrile/0.1N acetic acid (7:3), V_R=3.2 ml.

The acetyl derivatives

The non-labeled compound was prepared by acetylation of dibromo-salicylic acid according to the procedure of Walden and co-workers(1). This was used as

the standard for Prep-scale TLC separation of ^{82}Br-bromo-aspirin and for analyses of radioactivity in urine. Labeled ^{82}Br-dibromo-aspirin was prepared by acetylation of 3,5-Br-82-dibromosalicylic acid after vacuum evaporation from the previous procedure. To the residue, 100 μl of acetic anhydride/conc. H_2SO_4 was added. The acetylation was allowed to proceed for 15 minutes. Prep-scale TLC was carried out to separate acetyl-3,5-Br-82-dibromo-salicylic acid, using the same system as described previously. R_f of ^{82}Br-dibromo-aspirin was 0.7 as confirmed by an authentic sample of dibromo-aspirin. The labeled compound was extracted with ethanol for biodistribution studies. The unlabeled product and the purified ^{82}Br-dibromo-aspirin were also checked by TLC Silica Gel 60 F254 on aluminum with ethyl acetate/isopropanol/10% ammonia (5:2:1) R_f=0.68, and HPLC: μBondapak C-18 with an eluant of acetonitrile/0.1N acetic acid (4:6) V_R=9ml. This procedure was also repeated several times to afford non-radiolabeled acetyl-3,5-dibromo-salicylic acid for NMR, M.P. and elemental analysis.

Biodistribution Studies.

Studies of the Br-82 labeled acetyl-3,5-dibromo-salicylic acid were carried out in male A/J mice with and without neuroblastoma (to study uptake in major organs and tumor), and in rabbits to study the distribution between plasma and red blood cells, and in sterile inflammatory lesions. Five to 8 weeks old male A/J mice (Jackson Laboratory Bar Harbor, MA) underwent tumor transplant with C-13 neuroblastoma (frozen ampoule kindly supplied by National Cancer Institute through Mason Research Laboratory). The tumor was transplanted twice, each after 2-3 weeks growth. The tumors were about 1 cm and weighed 1-1.5 gm. Control studies were carried out with normal mice of the same age. Tail vein injection was carried out by using 1 μCi (4 μg/0.1 ml) of^{82}Br-dibromo-aspirin in ethanol/saline (1:1). The needle and syringe were checked for radioactivity before and after the injection. Four to five mice with

and without tumors were each sacrificed at 1, 2, 4 and 24 hours. Blood and urine samples were collected immediately. Urine was then analyzed by TLC with the aid of an authentic sample of dibromo-aspirin using Silica Gel 60F 254 on aluminum (E. Merck), and chloroform/ethanol/acetic acid (27:3:3) as solvent. Tissues were removed, blotted, weighed and counted in a gamma well counter. The activities were compared with a standard, and background corrected. The distribution of activity was expressed in % dose/organ and % dose/gram. The developed urine chromatograms were also assayed for activity, in accounting for the percentage of ^{82}Br present.

Sterile inflammatory lesions were induced in clean areas of the hind limb of rabbits, by using turpentine according to the procedure of Gelrud and co-workers(7). Subcutaneous injection into the clean area was carried out with 0.2 ml of turpentine, while injection of saline was made on the opposite limb of the same rabbit for control purposes. After 24 hours, two rabbits were administered 13 μCi of ^{82}Br-dibromo-aspirin through the ear vein, another rabbit was injected with 12 μCi of $K^{82}Br$ for a control study. The rabbits were sacrificed 2 hours later, since sterile inflammatory lesions have been reported to visualized with ^{67}Ga at this time period. Blood and urine samples were collected. The blood was centrifuged in order to separate the plasma from RBC. The tissues, inflammatory lesions, and the "control tissue" from the opposite rabbit limb, were removed, weighed, and counted in a gamma well counter. The distribution was expressed as % dose/organ and % dose/gram.

RESULTS

Radio-chemical yield of the labeled compounds was 0.2 to 0.4 mCi (4 to 8%). Total synthesis time was about 6 hours. Analysis of the products of Br-82-aspirin synthesis revealed three radioactive peaks with retention volumes of 4, 9 and 17 ml. The labeled aspirin eluted at 9 ml, as confirmed by an au-

thentic sample of dibromo-aspirin. TLC analysis of purified 3,5-Br-82-dibromosalicylic acid and Br-82-dibromo-aspirin showed a 95% radiochemical purity. Similarly prepared dibromo-aspirin underwent NMR, M.P. and elemental analysis. The NMR spectrum: $\delta_{TMS}(CDCl_3)$ 2.4 (singlet, 3H, CH_3), 7.8 (doublet, 1H, aromatic), 8.0 (doublet, 1H aromatic). The M.P. was 150-152°C . Elemental analysis (Schwarzkopf Microanalytical, Woodside, N.Y.) for $C_9 H_6 Br_2 O_4$ yielded; calculated C 31.95, H 1.77, Br 47.33, found C 31.98, H 1.79, Br 47.05.

The distribution of bromine-82 activity in normal and tumor mice is shown in Tables 1 and 11. For the normal mice, the tissues with high uptake of percent dose per gram at 1 hour were blood, 23%; lungs, 10% heart, 8%; kidneys, 6%; liver, 5% and spleen, 4%. The activities were lower at 24 hours. Distribution in the tumor mice was approximately similar to that of normal mice. The uptake in tumor was 6.3% per gram at 1 hour, and was lowered slightly to 5.4% at 24 hours. However, the tumor/blood ratios were always less than one. The urine analyses by TLC revealed that only 20 to 30% of activity in the urine was due to ^{82}Br-dibromo-aspirin. The majority of the activity was at a chromatographic spot corresponding to the bromide ion (no migration) with a small intermediate spot.

Table 111 shows the distribution of Br-82 activities in three rabbits with sterile inflammatory lesions. ^{82}Br-dibromo-aspirin was administered to the first two rabbits, while K^{82}Br was administered to the third. Distribution of the radioactivity was slightly different. The RBC/plasma ratio was 0.44 and 0.42 for ^{82}Br-dibromo-aspirin, and 0.83 for the K^{82}Br-study. The inflammatory/normal uptake ratios (per gram) were 1.04 and 1.91 for the ^{82}Br-dibromo-aspirin studies and 1.92 for the K^{82}Br.

Table I. Distribution of Br-82 radioactivity after I.V. administration of ^{82}Br-dibromo-aspirin into A/J mice at various time intervals (4 to 5 mice for each time interval).

	1 Hour		2 Hours		4 Hours		24 Hours	
Organ	%D/Organ	%D/Gram	%D/Organ	%D/Gram	%D/Organ	%D/Gram	%D/Organ	%D/Gram
Blood	40.04±4.24	23.08±1.80	35.63±4.02	21.92±2.59	29.93±0.68	17.90±0.53	15.36±6.73	14.60±3.72
Liver	5.05±0.91	4.80±0.52	4.74±0.96	4.48±0.14	5.71±0.43	5.49±0.87	3.06±1.52	2.38±1.02
Spleen	0.23±0.05	3.92±1.27	0.22±0.03	3.85±1.20	0.19±0.03	4.03±1.05	0.15±0.07	1.86±0.50
Kidneys	2.10±0.63	6.44±1.69	2.21±0.14	7.05±1.10	2.47±0.22	7.39±1.00	1.36±0.40	3.40±1.01
Heart	0.69±0.07	8.23±1.78	0.69±0.72	7.11±0.92	0.72±0.13	7.27±1.26	0.65±0.33	4.84±1.56
Lungs	1.11±0.21	10.08±2.24	1.54±0.45	11.79±1.98	1.74±0.30	13.75±3.03	0.85±0.18	5.84±1.28
Brain	0.20±0.04	0.70±0.08	0.22±0.05	0.89±0.15	0.27±0.06	1.16±0.33	0.26±0.11	0.76±0.33

Table 11. Distribution of Br-82 radioactivity after I.V. administration of ^{82}Br-dibromo-aspirin into A/J neuroblastoma mice at various time intervals. (4 to 5 mice for each time intervals).

	1 H		2 H		4 H		24 H	
Organ	%D/Organ	%D/Gram	%D/Organ	%D/Gram	%D/Organ	%D/Gram	%D/Organ	%D/Gram
Blood	37.26±0.79	21.31±0.76	31.90±5.54	19.75±2.23	33.72±1.99	21.31±2.08	20.00±5.20	11.51±2.44
Liver	3.20±0.16	3.08±0.38	4.53±1.19	4.05±1.33	3.09±0.15	3.44±0.14	3.42±0.84	3.14±0.50
Spleen	0.28±0.08	2.34±0.32	0.26±0.07	2.82±0.29	0.20±0.04	2.76±0.64	0.21±0.07	1.73±0.37
Kidneys	1.71±0.37	5.95±1.25	1.69±0.36	5.84±1.06	1.62±0.23	5.76±1.15	1.36±0.37	4.20±1.01
Heart	0.71±0.13	5.54±1.15	0.39±0.01	4.09±0.95	0.52±0.12	6.86±2.52	0.47±0.12	4.07±1.78
Lungs	1.04±0.26	7.60±1.63	1.48±0.58	8.30±1.03	1.21±0.21	9.61±1.87	1.08±0.24	6.26±0.71
Brain	0.21±0.03	0.94±0.31	0.24±0.12	1.29±0.26	0.18±0.06	0.82±0.14	0.20±0.12	0.61±0.40
Tumor	4.83±2.02	6.34±2.47	4.11±0.93	5.81±1.40	6.56±0.57	6.49±1.45	4.11±0.89	5.42±0.10

Table III. Distribution of Br-82 radioactivity 2 hours after I.V. administration of ^{82}Br-dibromo-aspirin into rabbit I and II, and K^{82}Br into rabbit III.

Organ	Rabbit 1		Rabbit II			Rabbit III
	%D/Gram	%D/Organ	%D/Gram	%D/Organ	%dose/gm	%dose of organ
Blood	0.105	11.681	0.071	7.444	0.171	17.187
Liver	0.027	1.252	0.024	1.191	0.052	2.752
Spleen	0.021	0.026	0.017	0.027	0.079	0.071
Kidneys	0.165	1.901	0.110	1.319	0.082	0.898
Heart	0.037	0.152	0.027	0.081	0.064	0.128
Lungs	0.046	0.382	0.030	0.182	0.110	0.833
Brain	0.003	0.018	0.002	0.011	0.040	0.262
Stomach	0.008	0.528	0.011	0.761	0.131	8.011
Small Intestine	0.015	0.633	0.012	0.613	0.076	4.972
Large	0.007	1.070	0.008	1.179	0.031	3.045
Skin Inflammatory	0.064	0.025	0.053	0.027	0.165	0.095
Muscle	0.031	0.042	0.035	0.016	0.171	0.170
Normal Skin	0.065	0.022	0.038	0.009	0.134	0.067
Muscle	0.026	0.029	0.008	0.006	0.041	0.026
RBC/PLASMA	0.44		0.42		0.83	
Inflam/NORMAL	1.04		1.91		1.92	

DISCUSSION

Since acetyl-3,5-dibromo-salicylic acid acetylates amino groups of intracellular hemoglobin in vitro, this study was undertaken to synthesize the radioactive analog in order to explore possible applications in hematology. Since ^{77}Br may become more available in the future(8), radiobromine labeled pharmaceuticals could play an increasing role in diagnostic studies. Therefore, we developed a procedure for synthesizing acetyl-3,5-Br-82-dibromo-salicylic acid (^{82}Br-dibromo-aspirin). The radiochemical yield was low ($\leq 8\%$), but the total synthesis time was only 6 hours.

Biodistribution of ^{82}Br-dibromo-aspirin in normal and tumor mice showed high activity in blood (23% dose/gram) at 1 hour. A similar study using rabbits revealed red blood cell/plasma ratios of 0.42 to 0.44 at 2 hours, indicating that binding to red blood cells was small. Since aspirin is also an anti-inflammatory and antiprostaglandin agent, uptake of activity in neuroblastoma (mice) and sterile inflammatory lesions (rabbits) was also investigated. Tumor uptake ranged from 6.3% dose/gram at 1 hour to 5.4% dose/gram at 24 hours. However, the tumor/blood ratio at 1 hour was only 0.30. The inflammatory/normal uptake per gram was 1.04 and 1.91 in two rabbits. For control study with $K^{82}Br$, this ratio was 1.92. Analysis of urine from the ^{82}Br-dibromo-aspirin mice revealed that only 20 to 30% of the radioactivity was due to the injected compound. This low percent was due to dehalogenation processes in vivo as shown by the majority of activity migrating similarly to the bromide ion. A small percentage of the activity was attributed to unidentified radiolabeled metabolites. The dehalogenation of labeled dibromo-aspirin will prevent use of the compound diagnostically.

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